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Research paper

Diversity of rickettsiae in a rural community in northern California

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ABSTRACT

Far northern California forests are highly biodiverse in wildlife reservoirs and arthropod vectors that may propagate rickettsial pathogens in nature. The proximity of small rural communities to these forests puts people and domestic animals at risk of vector-borne infection due to spillover from wildlife. The current study was conducted to document exposure to rickettsial pathogens in people and domestic animals in a rural community, and identify which rickettsiae are present in sylvatic and peri-domestic environments near this community. Blood samples from people, domestic animals (dogs, cats, and horses) and wild carnivores were tested for *Rickettsia* spp. antibodies and DNA (people and domestic animals only) by serology and real time (RT)-PCR, respectively. Ectoparasites were collected from dogs, wild carnivores and from vegetation by flagging, and tested for *Rickettsia* spp. DNA by RT-PCR. DNA sequencing of the rickettsial 17 kDa protein gene or the *ompA* gene was used for species identification. Despite a seroprevalence of 3% in people, 42% in dogs, 79% in cats, 33% in gray foxes, and 83% in bobcats, RT-PCR on blood was consistently negative, likely because the sensitivity of this test is low, as *Rickettsia* spp. do not often circulate in high numbers in the blood. *Rickettsia* spp. DNA was found in four flea species collected from bobcats and *Ctenocephalides felis* collected from domestic dogs. All amplicons sequenced from fleas were *R. felis*. *Ixodes pacificus* collected by flagging were commonly infected with a *Rickettsia* sp. endosymbiont. *Rickettsia rhipicephali* DNA was found in *Dermacentor variabilis* from dogs, black bears, a gray fox, and a *D. occidentalis* collected by flagging. *Dermacentor variabilis* from dogs and black bears also contained *R. montanensis* DNA. Multiple *Rickettsia* spp. (including species with zoonotic and pathogenic potential) were found among human biting arthropod vectors of both wild and domestic carnivores and on flags. Knowledge of the diversity of *Rickettsia* spp. that are present within arthropod vectors to which people and domestic animals are exposed is an essential first step is making an accurate diagnosis and in better understanding the epidemiology of these potential pathogens. Within-host and vector interaction among these species may play a role in spillover into human and domestic animals.

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1. Introduction

Rickettsiae are obligately intracellular, pleomorphic bacteria that can cause disease in humans and other animals (Parola et al., 2005). These organisms can be transmitted by arthropod vectors (e.g. fleas, ticks, mites, and lice) to their mammal hosts. Various classifications have been proposed, but recently rickettsiae have been organized into four groups: the pathogenic spotted fever group (SFG) and typhus group, and the non-pathogenic *Rickettsia bellii* group and *Rickettsia canadensis* group (Merhej and Raoult, 2011). Multiple species of SFG rickettsiae are found in California, including *R. rickettsii* (the causative agent of Rocky Mountain spotted fever), *R. philippi, R. rhipicephali, R. felis* and *R. typhi* (Parola et al., 2005). Rickettsial infections can be fatal (even in otherwise healthy individuals), despite the availability of effective treatment, due to

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diagnostic challenges and delayed treatment (Bakken et al., 2006; Palmer and Azad, 2012).

Despite surveillance and research programs in many states targeting human cases and to a lesser extent testing of wildlife and vectors, numerous aspects of sylvatic cycles of these diseases remain poorly understood. These cycles comprise a high diversity of wildlife species and various pathogen strains, thus allowing opportunities for co-infection, and host-generalist and specialist vectors. The forests of northern California are highly biodiverse, providing habitat for numerous wildlife and arthropod reservoirs that maintain sylvatic cycles of rickettsial pathogens. In addition, many residents of rural northern California suffer from health disparities including lower socioeconomic status, being under-served minorities, and having limited access to health care. These same residents may also have high exposure to pathogen vectors through both occupation (forestry, agriculture, etc.) and recreation (hunting, fishing, hiking, camping). Early symptoms of vector-borne diseases are often mild or nonspecific, and this can lead to delayed, or misdiagnosis, even in at-risk communities.

We performed a cross-sectional epidemiologic study to determine exposure to rickettsial pathogens in people, domestic animals and wild carnivores in a rural community. In addition, we tested arthropod vectors (including human-biting ticks and fleas) collected from domestic dogs and wildlife to determine which rickettsiae are present in the sylvatic and peri-domestic environments near this community in Humboldt County, California, and could pose a risk to humans and domestic animals. By determining the presence of and exposure to multiple rickettsiae, we can better understand their impact on human and animal health in this rural northern California community and help pave the way for more effective surveillance, prevention, control and diagnostic measures.

2. Methods

2.1. Study area

Humboldt County is a densely forested, mountainous, rural county located in northern California along the coastline. Elevations range from sea level along the Pacific coast to 1170 m in the nearby mountain ranges. Vegetation in forested areas primarily consists of redwood (*Sequoia sempervirens*), Ponderosa pine (*Pinus ponderosa*), Douglas fir (*Pseudotsuga menziesii*), tanoak (*Notholithocarpus densiflorus*), madrone (*Arbutus menziesii*), Oregon white oak (*Quercus garryana*), California black oak (*Quercus kelloggii*), evergreen huckleberry (*Vaccinium ovatum*), tobacco brush (*Ceanothus velutinus*), salal (*Gaultheria shallon*), and poison oak (*Toxicodendron diversilobum*). The non-forested areas include residential areas, natural prairies, large rock outcrops, and costal beaches. Forestry is a major industry in Humboldt County: Humboldt is the largest timber-producing county in California, responsible for almost 20% of all timber production in the state (Laaksonen-Craig et al., 2003).

Humboldt County includes eight Native American reservations (United States Census Bureau, 2010) and several areas are designated medically underserved including the Arcata, Ferndale, Garberville, Redway, Mckinleyville, and North Coastal service areas (Healthcare Workforce Development Division, 2010). 19.5% of the population of Humboldt County was living below the poverty level in 2000 compared to 14.2 statewide and 12.4 nationally (VanArsdale and Barry, 2008). The median household income for the county was 42,153 in 2010 compare to 61, 489 for California (United States Census Bureau, 2010).

2.2. Participant recruitment and sampling

Participants and their domestic animals were recruited throughout Humboldt County, California by word-of-mouth and by posting flyers in visible, high-traffic areas. During previous fieldwork and interactions with residents of Humboldt County, several groups of individuals had expressed a concern for their exposure to ticks and potential tick-borne diseases and an effort was made to include them in the study. These groups included forestry workers, wildlife biologists, college students interested in wildlife research, and Native Americans living in close proximity to forested areas. Recruitment events were scheduled to coincide with other community activities where participants were also recruited using a script. After receiving informed consent from participants, basic demographic information and whole blood samples were collected by a licensed nurse. In conjunction, veterinarians and veterinary students collected blood samples and ectoparasites from participant's dogs, cats, and horses as part of no-cost rural area veterinary clinics offering routine veterinary care and vaccinations. Blood samples and ectoparasites were collected from wild carnivores and from vegetation by flagging as part of various other studies. Collected ectoparasites were stored in 70% ethanol at room temperature. Blood samples were collected into sterile tubes containing EDTA and stored at 4 °C until plasma was separated and stored at −20 °C until testing. All work was performed under appropriate guidance and permits from the University of California, Davis Institutional Review Board, Institutional Animal Care and Use Committee and the California Department of Fish and Wildlife scientific collection permits.

2.3. Blood DNA extraction and real time-PCR

DNA was extracted from 100 μl of whole blood using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the blood, spin-column protocol. A sensitive and specific real time (RT)-PCR assay for the detection of *Rickettsia* genus DNA (including both SFG and typhus group) was performed as described (Stenos et al., 2005) (Table 1). Samples were considered positive if the threshold cycle (CT) <40 with a characteristic amplification curve. Three negative water controls and a sequence-confirmed *R. prowazekii* positive control were included in each run.

2.4. Serology

Indirect immunofluorescence assays (IFA) were performed for antibodies to *R. rickettsii* on all human and domestic animals and on wild carnivores when a blood samples was available. Due to cross-reactivity, positive results are not considered species-specific (Bakken et al., 2006). Plasma was diluted in phosphate-buffered saline (PBS) at 1:64 and applied to commercial slides (VMRD, Pullman, WA). Slides were incubated at 37 °C with moisture for 30 min and washed three times with PBS. They were then incubated for 30 min with fluorescein-labeled, immunoglobulin G heavy and light chain conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted in PBS at 1:100 for humans and domestic animals. Wherever possible, we used species-specific secondary antibodies. For wildlife for which species-specific antibodies were not available, anti-dog conjugate was used for gray fox and raccoons, and for bobcats we used anti-cat conjugate following the same procedure. Slides were washed three additional times and counter-stained with eriochrome black. Positive and negative control serum was included in each batch. Samples were considered positive if they had strong fluorescence detected compatible with the morphology of the antigen on the slide.

2.5. Ectoparasite identification, DNA extraction, and real-time PCR

Ticks were identified to species and sex using California taxonomic keys (Furman and Loomis, 1984). DNA was extracted from

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Table 1
Oligonucleotide primer and probe sequences for PCR assays on ticks and fleas collected from wildlife, domestic animals, and by flagging in Humboldt County, California.

Function	Target gene	Name	Primer sequence 5′ – 3′	PCR type	Product size (bases)	Refs.
Screening	Citrate synthase	**CS-F**	TCG CAA ATG TTC ACG GTA CTT T	**Real time-PCR**	74	Stenos et al. (2005)
		CS-R	TCG TGC ATT TCT TTC CAT TGT G	–		
		CS-P	TGC AAT AGC AAG AAC CGT AGG CTG GAT G	**Probe**		
Ticks	Rickettsial outer membrane protein A	**R190-70**	ATGGCGAATATTTCTCCAAAA	**Primary PCR**	532	Regnery et al. (1991)
		R190-602	AGTGCAGCATTCGCTCCCCCT	–		
Fleas	17 kDa protein gene	**R17-122**	CAGAGTGCTATGAACAAACAAGG	**Primary PCR**	246	(Anderson and Tzianabos, 1989; Tzianabos et al., 1989)
		R17-500	CTTGCCATTGCCCATCAGGTTG	–		
		TZ-15	TTCTCAATTCGGTAAGGGC	**Nested PCR**		
		TZ-16	ATATTGACCAGTGCTATTTC	–		
Fleas	17 kDa protein gene	**17kD1**	GCTCTTGCAACTTCTATGTT	**Primary PCR**	434	Webb et al. (1990b)
		17kD2	CATTGTTCGTCAGGTTGGCG	–		

ticks using NH_4OH as previously described (Foley and Piovia-Scott, 2014). Flea identification was performed after body contents were removed for DNA extraction. A dorsal incision was made over the central tergites with a sterile scalpel (Brigham Young University, 2012). The flea body was then incubated overnight in ATL buffer and proteinase K (Qiagen) at 55 °C, the exoskeleton removed, and DNA was extracted from the remaining digested material using a DNeasy Blood and Tissue Kit (Qiagen) following manufacturer's guidelines. Flea exoskeletons were cleared by incubating in dilute KOH for 24 h, then dehydrated in an ethanol series (75, 85, 95, and 100% for 30 min each), and mounted in Euparal (BioQuip, Rancho Dominguez, California, USA). Fleas were identified to sex and species using western North American taxonomic keys, except *Pulex* spp. females for which the species cannot be distinguished morphologically (Stark, 1958; Hubbard, 1958; Lewis et al., 1988). RT-PCR for detection of *Rickettsia* spp. DNA (as described above) was performed on all ectoparasites individually except for *Ixodes pacificus,* which were tested in pools of five to conserve resources as they are commonly infected by a rickettsial endosymbiont.

2.6. Conventional PCR and sequencing

Conventional PCR for amplification of the rickettsial outer membrane protein A gene (*ompA*) gene was performed on all *Dermacentor* spp. ticks that were positive by RT-PCR, and five *I. pacificus* ticks from the pool that had the lowest CTs (Table 1). Primers R190-70 and R190-602 were used for amplification as described (Anstead and Chilton, 2013). Amplification of the 17 kDa protein gene was performed on the ten *Ctenocephalides felis* DNA samples that were positive by RT-PCR with the lowest CT and all non-*C. felis* flea species that were positive by RT-PCR. Amplification was performed using either a nested PCR protocol with external primers R17–122 and R17-500 followed by internal primers TZ15 and TZ16 or single round PCR with primers 17kD1 and 17kD2 (Shapiro et al., 2010; Webb et al., 1990a). PCR reactions were performed using GoTaq Green Master Mix (Promega, Madison, WI, USA) per manufacturer instructions. Each 25 μl reaction contained 1.0 M of each primer and 3 μl of template DNA for the first round and 1 μl of the first round product for the nested protocol. Thermal cycling conditions were as originally described (Anstead and Chilton, 2013; Paddock et al., 2008; Shapiro et al., 2010; Stenos et al., 2005; Webb et al., 1990b). Sequence-confirmed positive and water-containing negative control reactions were included in each run. Results of PCR were assessed by electrophoresis and UV-transillumination of GelStar (Lonza, Rockland, ME, USA) stained 1% agarose gels. Bands of the expected size were excised and cleaned with a QIAquick gel extraction kit (Qiagen) per manufacturer instructions. Products were sequenced in the forward direction in an ABI Prism 3730 Genetic Analyzer (UC DNA Sequencing Facility, Davis, CA, USA).

2.7. Sequence analysis and statistical methods

Sequences were manually trimmed and corrected if the nucleotide could be unambiguously determined, then aligned using CLC Main Workbench 6 (CLC bio, Watham, MA, USA). Sequences from fleas were compared to *R. felis* (Acc# CP000053) using BLAST analysis (GenBank, NCBI, Bethesda, MD, USA). Sequences from ticks were aligned using CLC. All unique sequences generated were submitted to GenBank for accession. Confidence intervals were calculated by inverting Rao's score test (Wilson, 1927). An estimate for prevalence of infection in *I. pacificus* based on pooled samples was calculated using the PooledInfRate version 4.0 Excel add-in (Biggerstaff, 2009). CTs from RT-PCR positive *C. felis* and non-*C. felis* fleas were compared using a Kruskal-Wallis rank sum test to determine if they were significantly different. Correlation between vector PCR-status and host sero-status was determined by calculating the phi coefficient. All calculations and analyses were performed using the software package R (R-Development Core Team, www.r-project.org).

3. Results

3.1. Vertebrate assay results

Blood samples were collected from 130 people, 163 dogs, 29 cats, 17 horses, 15 gray foxes, six bobcats and one raccoon from Humboldt County. All blood samples were tested for antibodies to *R. rickettsii:* 3% of people (4; 95% CI: 1–8%), 42% of dogs (69; 95% CI: 35–50%), 79% of cats (23; 95% CI: 60–92%), 33% gray foxes (5; 95% CI: 13–61%), 83% bobcats (5; 95% CI: 36–99%), and no raccoons or horses (95% CI: 0–95%; 95% CI: 0–20%, respectively) were seropositive by IFA at 1:64. None of the human or domestic animal blood samples were positive for *Rickettsia* spp. DNA by RT-PCR; wildlife blood samples were not tested by RT-PCR.

3.2. Ectoparasites

Ectoparasites collected from dogs included 48 *D. variabilis*, 52 *C. felis,* three *C. canis,* and five *Pulex* spp. (60 fleas total), while no ectoparasites were collected from cats or horses (Table 2). Additionally, 56 *Cediopsylla inaequalis,* 17 *Orchopeas cascadensis,* four *Odontosyllis dentatus* and one *Pulex* spp. were collected from bobcats, and 81 *Pulex* spp. and 38 *D. variabilis* were collected from gray foxes. *D. variabilis* were also collected from black bears (146)

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Table 2
Summary of arthropod vectors collected from wildlife and domestic animal hosts and by flagging in Humboldt County, California and tested for *Rickettsia* spp. DNA by RT-PCR.

Host	Number positive	Number tested	Percent positive
Dog *(Canis lupus familiaris)*			
Ctenocephalides canis	0	3	0
C. felis	20	52	38.5
Pulex spp.	0	5	0
Dermacentor variabilis	5	48	10.4
Bobcat *(Lynx rufus)*			
Cediopsylla inaequalis	3	56	5.3
Odontosyllis dentatus	2	4	50
Orchopeas cascadensis	1	17	5.9
Pulex sp.	1	1	100
Gray Fox *(Urocyon cinereoargenteus)*			
Pulex spp.	0	81	0
D. variabilis	1	38	2.2
Black Bear *(Ursus americanus)*			
D. variabilis	8	146	5.5
Raccoon *(Procyon lotor)*			
D. variabilis	0	3	0
Flag			
D. occidentalis	2	9	22.2
D. variabilis	0	80	0

and raccoons (3). *D. occidentalis, D. variabilis* and *I. pacificus* were collected by flagging (9, 80 and 61, respectively).

Twenty of the 52 (39%) *C. felis* collected from domestic dogs and 7 (9%) of the 78 fleas collected from bobcats, including at least one of each flea species that was identified, were positive for *Rickettsia* spp. DNA by RT-PCR, but none of the 81 *Pulex* spp. collected from gray foxes were PCR-positive (Table 2). CT values from RT-PCR between *C. felis* (mean CT: 25.75) and non-*C. felis* fleas (mean CT: 36.22) were significantly different ($X^2 = 9.408$, $p = 0.002$), which shows that *C. felis* contained more *Rickettsia* spp. DNA than non-*C. felis* fleas. Two of the 9 (22%) *D. occidentalis* and 14 of the 312 (4%) *D. variabilis,* and 11 of the 12 pools (prevalence: 34.7%; 95% CI: 19.5–65.5%) of *I. pacificus* collected by flagging were PCR-positive for *Rickettsia* spp. The phi coefficients for correlation between flea or tick PCR-status and host sero-status were −0.04 and 0.07, respectively.

3.3. Sequencing and species identification

Sequencing of the 17 kDa gene from flea DNA samples resulted in ten sequences from *C. felis* collected from domestic dogs, and two from *C. inaequalis*, two from *O. dentatus*, and one from *O. cascadensis* collected from bobcats. Sequences were either 368 (n = 6) or 285 (n = 9) nucleotides in length depending on PCR protocol used (Table 1) and were genetically invariant. All sequences were 100% identical to *R. felis* strain California 2 (GenBank Acc# CP000053) (Ogata et al., 2005).

Sequencing of the *ompA* gene from ticks resulted in 12 sequences from *D. variabilis*, one from a *D. occidentalis* and four from *I. pacificus*. Sequencing failed on two amplicons obtained from *D. variabilis* and one from a *D. occidentalis*. All sequences from *I. pacificus* were 464 nucleotides in length and were identical to each other and to an unpublished sequence in GenBank of an *I. pacificus* endosymbiont (Acc# GU047354). Eight of the sequences from *D. variabilis* (three collected from domestic dogs and five from black bears) were 472 nucleotides in length, were identical (Genotype A), and showed a 98% nucleotide-sequence identity to *Rickettsia montanensis* str. OSU 85–930 (Acc# CP003340) on BLAST analysis. Four of the sequences from *D. variabilis* (two collected from black bears, one from a domestic dog, and one from a gray fox) were 466 nucleotides in length, were identical (Genotype B) and BLAST analysis of the sequence showed a 99% nucleotide sequence identity to *R. rhipicephali* strain HJ#5 (Acc# CP013133). The sequence from the *D. occidentalis* collected by flagging was 470 nucleotides (Genotype C) in length and BLAST analysis of the sequence showed a 99% nucleotide sequence identity to *R. rhipicephali* strain HJ#5 (Acc# CP013133). Nucleotide differences from reference sequences are shown in Fig. 1. Unique sequences were submitted to GenBank (Acc# KX449323-30).

4. Discussion

In this study, we documented that people, dogs, cats, gray foxes, and bobcats are exposed to rickettsiae based on the presence of antibodies to *R. rickettsii*. Examination of arthropod vectors led to successful identification of multiple *Rickettsia* spp., including zoonotic pathogens in human-biting vectors. DNA from the human pathogen *R. felis* was commonly present in *C. felis* collected from domestic dogs. *C. felis* is a human-biting flea commonly found on dogs and cats and is a biological vector of this pathogen. *R. montanensis* and *R. rhipicephali*, both species of unknown pathogenicity, were detected in *Dermacentor* spp. ticks collected from domestic dogs, black bears, a gray fox, and by flagging.

In addition to species of unknown pathogenicity, a *Rickettsia* spp. endosymbiont was detected in *I. pacificus* collected by flagging. The role of this endosymbiont not fully understood though studies suggest that it may be beneficial for the tick by providing de novo folate biosynthesis (Hunter et al., 2015; Kurlovs et al., 2014). *I. pacificus* is known to feed on humans (Furman and Loomis, 1984; Spencer, 1963) and is the main vector of Lyme disease and human granulocytic anaplasmosis in the western US (Foley et al., 2004; Lane et al., 2005; Peavey et al., 2000). Endosymbiotic species may benefit the vector or compete within the vector with other tick-borne pathogens (Hotopp et al., 2006; Macaluso et al., 2002). It is unknown at this time what role this endosymbiont plays in this system.

Both *R. rhipicephali* and *R. montanensis* fall within the SFG (which includes *R. rickettsii*, the causative agent of Rocky Mountain spotted fever) and are closely related to one another (Merhej and Raoult, 2011). *R. rhipicephali* was first isolated from *Rhipicephalus sanguineus* but has also been detected in *D. occidentalis*, *D. andersoni* and *D. variabilis* in the United States (Wikswo et al., 2008).

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R. montanensis
Nucleotide   2          54        64          85          115         153         190         393
CP003340    CCGCT-[45]-GCATTCGGTGTTGTTGC-[24]-CTAATAA-[23]-CTGCTGC-[31]-GTAACTA-[30]-ATGTGGC-[196]-GCTATTG
GenotypeA   .T...      ...C.........C...      ...C...     ...T...     ...G...     ...G...     ...G...

R. rhipicephali
Nucleotide     49          107         190         254         326         362
CP013133    -GCGTGGC-[51]-TGACTTG-[76]-GTACGGC-[57]-GGCTATA-[65]-GACAGGA-[29]-CGGCAAA-
GenotypeB   -...G...       .......     .......     ...T...     ...G...     ...T...
GenotypeC   -...G...       ...T...     ...T...     ...C...     ...G...     ...C...
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Fig. 1. Nucleotide differences between reference ompA sequence of *R. montanensis* (CP003340) and *R. rhipicephali* (CP013133) from GenBank and sequences obtained from ticks collected from wildlife, domestic animals and by flagging in Humboldt County, California. Matching bases are shown as dots. Sequences are abbreviated in sections where no differences occur and the number of bases abbreviated is shown in brackets. Genotype A was detected in eight *D. variabilis*; three collected from domestic dogs and five from black bears. Genotype B was detected in four *D. variabilis*, two collected from black bears, one from a domestic dog, and one from a gray fox. Genotype C was detected in a *D. occidentalis* collected by flagging.

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While there has been no evidence of *R. rhipicephali* causing disease in humans, experimental infection in meadow voles caused moderately severe disease (Burgdorfer et al., 1975). *R. montanensis* is present in *Dermacentor* spp. across North America and there are no confirmed cases of human disease caused by this SFG rickettsia. However, recently an afebrile child with a rash-associated disease developed *R. montanensis*-reactive antibodies after being bitten by a *D. variabilis* tick that tested positive for *R. montanensis* DNA by PCR (McQuiston et al., 2012). In addition to potential pathogenicity, these species could theoretically "immunize" mammal host species against more pathogenic SFG species by causing the production of cross-protective antibodies (Fairlie-Clarke et al., 2009). Additionally, Macaluso et al. (2002) showed that infection of *D. variabilis* ticks with *R. rhipicephali* or *R. montanensis* inhibited the transovarial transmission of the second SFG rickettsia species. However, ticks are commonly found with mixed infections as well (Milhano et al., 2010; Nieto and Foley, 2009; Schicht et al., 2012), therefore the dynamics of co-circulating *Rickettsia* spp. are unclear.

Flea-borne spotted fever (also called cat-flea typhus) is caused by *R. felis*, and carried by several flea species, though it is most commonly found in the cat flea, *C. felis,* which readily feeds on humans (Azad et al., 1997). Infection with *R. felis* is most commonly through flea bites, which allow *R. felis* to enter the bloodstream and then infect endothelial cells where it replicates within the cytoplasm and causes generalized vasculitis (Raoult and Parola, 2007). Almost 40% of the *C. felis* collected from domestic dogs in this study were RT-PCR positive for *Rickettsia* spp. DNA and all ten samples that were sequenced were *R. felis*. Additionally, 3% of people, over 40% of dogs and almost 80% of cats tested were seropositive for antibodies to *R. rickettsii*, which could represent reactivity of *R. felis* antibodies. The high proportion of infected fleas in this study that are infesting domestic dogs suggests that *R. felis* may be an important public health concern in this community and that human cases are potentially being mis- or under-diagnosed.

In addition to *C. felis*, *R. felis* DNA was present in three other flea species collected from bobcats as well. CTs from RT-PCR for *Rickettsia* spp. were lower in *C. felis* than non-*C. felis* fleas, indicating there was significantly more *R. felis* DNA in *C. felis* than in other flea species. Additionally, the phi coefficients revealed that there was no correlation between vector PCR-status and host sero-status for either ticks or fleas. Possible reasons for this finding include: seropositive hosts were not acutely infected (or rickettsemic) at the time they were fed on by vectors, some seropositive hosts could develop antibodies but not be competent hosts (i.e. not develop strong enough active infection to serve to infect a vector), or seronegative hosts could be a source for infecting a vector if they are acutely infected and had not yet developed antibodies.

In this study, our sampling of people, animals, and vectors was not standardized or random and therefore caution should be exercised in externally generalizing the findings. As this was a pilot study and we expected a low prevalence, we aimed to include people who had a high exposure to ticks through either occupation or recreation. We were unable to use species-specific conjugates for our IFA serology, as they are not commercially available; as is commonly done, we used the closest phylogenetically related domestic species for which a conjugate was available. The effect of this, if any, would be to decrease the sensitivity of the assay.

Here we document the diversity of *Rickettsia* spp. found among arthropod vectors, including those infesting domestic animals and known to feed on humans. Additionally, our serologic data show that both humans and domestic animals are exposed to these bacteria. There are still many unanswered questions about the dynamics and transmission cycles responsible for sylvatic maintenance of these microbes and their impact on humans and domestic animals. This research is the first step in better understanding the epidemiology of these agents however more research is needed to understand within-host and vector interaction among these species and how they may play a role in spillover into humans and domestic animals.

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